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LETTER TO THE EDITOR

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There is inadequate evidence to support the division of the genus *Borrelia*

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There are surely scientific, genetic or ecological arguments which show that differences exist between the relapsing fever (RF) spirochaetes and the Lyme borreliosis (LB) group of spirochaetes, both of which belong to the genus *Borrelia*. In a recent publication, Adeolu and Gupta [1] proposed dividing the genus *Borrelia* into two genera on the basis of genetic differences revealed by comparative genomics. The new genus name for the LB group of spirochaetes, *Borreliella,* has subsequently been entered in the GenBank database for some species of the group and in a validation list (List of new names and new combinations previously effectively, but not validly, published) [2]. However, rapidly expanding scientific knowledge and considerable conflicting evidence combined with the adverse consequences of splitting the genus *Borrelia* make such a drastic step somewhat premature. In our opinion, the basis of this division rests on preliminary evidence and should be rescinded for the following reasons:

(1) The proposed split of the genus rests on differences in conserved signature indels (CSI) and conserved signature proteins (CSP) between LB and RF spirochaetes. A major omission in the study published by Adeolu and Gupta [1] is the exclusion of a *Borrelia* clade containing RF-like species that utilize hard ticks as vectors and reptiles as reservoir hosts [3, 4].

To identify proteins that are uniquely present in various groups of *Borrelia*, BLAST searches [5] were performed by Adeolu and Gupta [1] using each protein in the genomes of *Borrelia burgdorferi sensu stricto* (s.s.) B31^T and *Borrelia recurrentis* A1 as queries. Out of 1041 and 1390 protein coding genes (i.e. the number of proteins reported in GenBank accession numbers NC_011244 and NC_001318) present in *B. recurrentis* A1 and *B. burgdorferi* s.s. B31^T, respectively, 15 CSI (seven for LB, eight for RF) and 25 CSP (21 for LB, four for RF) were found to be unique for the respective groups. However, two of the four CSPs that are apparently unique for the RF group species are not found in all members of this group and therefore do not represent true signature proteins. Hence, just two CSPs and eight CSIs are unique to the RF group.

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The same holds true for the LB group of spirochaetes. Five of the 21 CSPs present only in the LB group of spirochaetes are not found in all species of the *B. burgdorferi sensu lato* (s.l.) complex. Furthermore, 12 of these CSPs are hypothetical proteins with unknown functions, and so this challenges the utility of these CSPs as unique signature proteins. These facts coupled with the omission of the entire clade of reptile-associated species (Fig. 1) underscore our criticism and highlights the uncertainty around the proposed genus split. Presumably this is only the tip of the iceberg, as more RF-like and LB species continue to be detected and described every few years [3, 6]. In this context, it is our opinion that it would be prudent to retain the generic name *Borrelia* for both LB and RF spirochaetes.

(2) The genus *Borrelia* is known to be cohesive because of the species shared spirochaetal morphology (with some variations within both groups such as the number of flagella, number and regularity of spirals), comparable genome structure, similar G+C content (nearly 30 %), and common vector-borne lifestyle (using ticks as vectors in natural

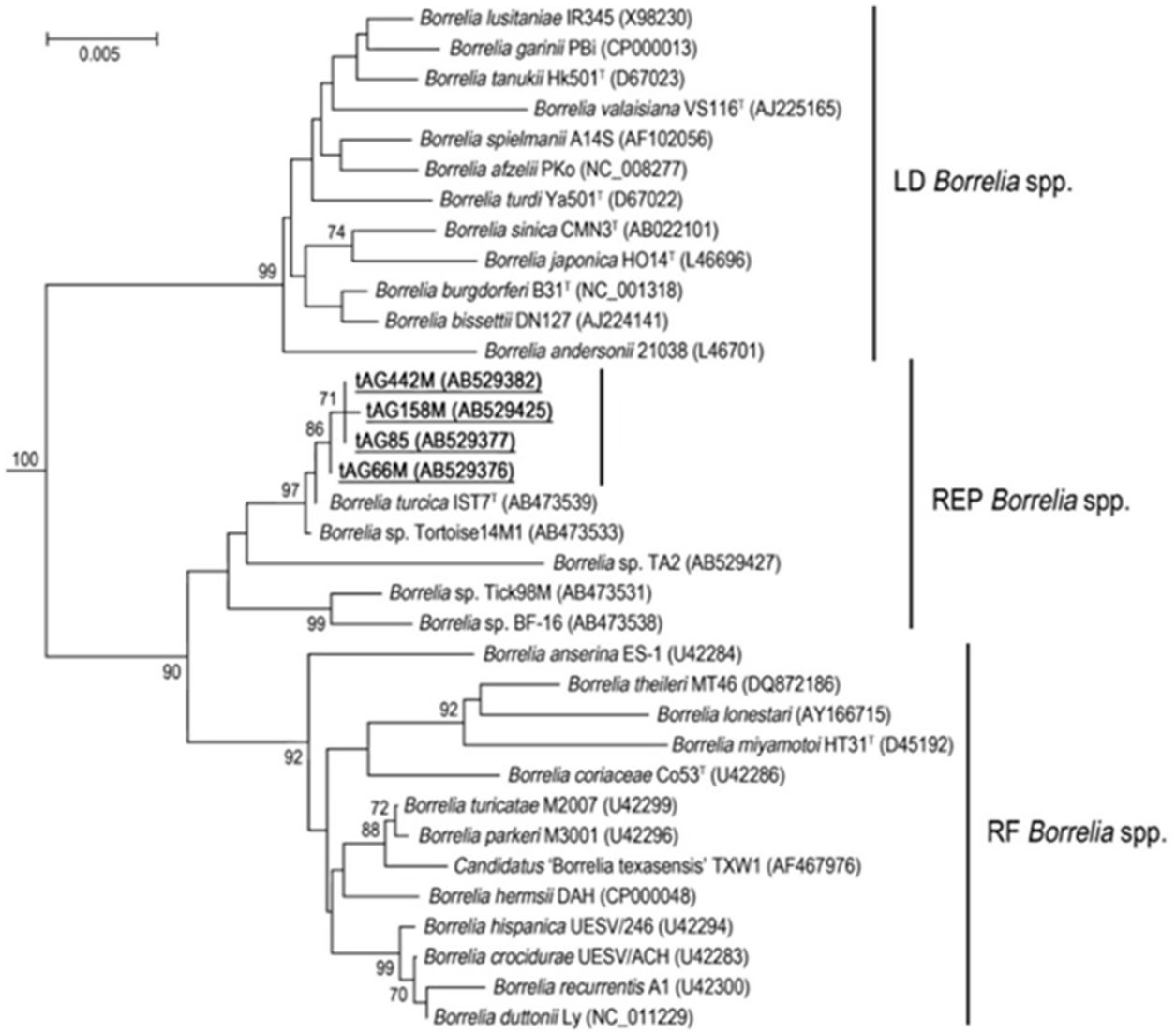

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences of the genus *Borrelia*. Phylogenetic trees were reconstructed based on neighbour-joining methods, and bootstrap tests were carried out according to Kimura's 2-parameter distances method. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (pairwise deletion option). There were a total of 1565 positions in the final dataset. The phylogenetic branches were supported in >70 % by the bootstrap analysis. *Spirochaeta americana* (GenBank accession number AF373921), *Treponema pallidum* (NC_000919) and *Cristispira* sp. (U42638) were used as outgroups. LD, Lyme Disease; REP, species of *Borrelia* using reptiles as reservoir hosts; RF, relapsing fever. Bar, 0.005 % sequence divergence. Figure modified from Takano *et al.* 2011, Environmental Microbiology Reports 3 (5), 632–637, and reproduced with permission of John Wiley and Sons.

transmission cycles, with one exception, *B. recurrentis* which is transmitted by *Pediculus humanus*) Initial work suggested that relapsing fever species are transmitted by soft ticks whilst species belonging to the *B. burgdorferi* s.l. species complex were transmitted by hard ticks [7]. This view had to be modified because several species of the genus *Borrelia* that cluster phylogenetically with RF spirochaetes were revealed to be transmitted by hard ticks. Importantly, *Borrelia miyamotoi* [8] which has been shown to cause an RF-like illness [9], referred to as hard tick relapsing-fever (HTRF [10]), is transmitted by hard ticks of the genus *Ixodes. B. miyamotoi* occurs sympatrically with LB group spirochaetes and, indeed, the four primary *Ixodes* spp. ticks that transmit *B. burgdorferi* s.l. spirochaetes to humans likewise are the principal vectors of *B. miyamotoi*. Further RF-like and LB spirochaetes are being discovered and described [3, 6], and the ecological and genetic differences between these groups will most certainly become even more blurred in the future.

Underpinning this point, we have performed a comparative genomic analysis that demonstrated the close genetic relationship between LB and RF group spirochaetes. MUMmer v. 3 [11] was implemented to align DNA sequences of the main chromosomes of the LB spirochaetes *B. burgdorferi* s.s. B31^T (GenBank accession number NC_001318.1), *Borrelia bavariensis* NMJW1 (NC_018747.1) and the RF spirochaete *Borrelia duttonii* Ly (NC_011229.1, a genetically more complete spirochaete than *B. recurrentis* used above). MUMmer is an ultrafast alignment tool and is designed to find exact matches for a minimum specified length (here, 20 bp being chosen) between two or more input sequences. Sequences were uploaded in fasta format and MUMmer was run using standard parameters.

Comparison of *B. bavariensis* NMJW1 (filled triangles) or *B. duttonii* Ly (filled diamonds) with *B. burgdorferi* s.s. B31^T resulted in nearly a straight line (from the bottom left to the top right) indicating a high degree of similarity between them (Fig. 2), and that no major rearrangement had occurred in either of the two strains compared to *B. burgdorferi* s.s. B31^T. For sake of clarity, only forward-sequence comparisons are shown. The dots scattered across the plot are matches of the minimum 20-bp sequence to other regions in the genome. Such 'mismatches' were found in both comparisons, i.e. *B. bavariensis* versus *B. burgdorferi* s.s., and *B. duttonii* versus *B. burgdorferi* s.s. (Fig. 2). We conclude that the genospecies compared here display a high degree of synteny.

(3) As for the clinical symptoms caused by species of the genus *Borrelia*, the symptomology that differentiates RF spirochaetes from the LB group of spirochaetes has been blurred by recent case descriptions. For example, a patient with clinical symptoms resembling those of Lyme

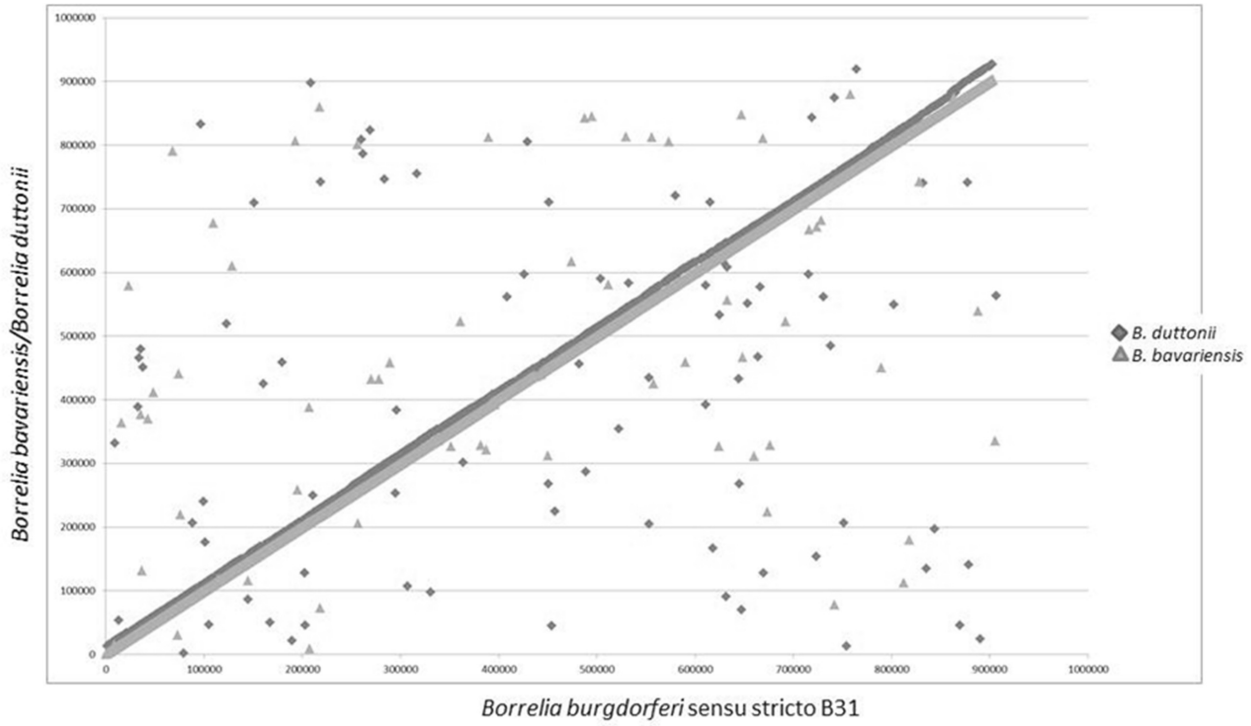

Fig. 2. Similarity dot plot (compiled in MUMmer v. 3) of the main chromosome of *B. duttonii* Ly (filled diamond) and *B. bavariensis* NMJW1 (filled triangle) compared to *B. burgdorferi* B31^T. The figure underlines the high similarity at the main chromosome of RF group spirochetes and LB group spirochetes.

neuroborreliosis was diagnosed as being infected with the RF group species *B. miyamotoi* [12]. Interestingly, infection with the recently described genospecies of the *B. burgdorferi* s.l. complex, *Borrelia mayonii*, produced high spirochaetal blood densities, akin to that seen following infection with species of the RF group [6].

Thus, splitting the genus does not provide any assistance as far as clinical evaluation is concerned. It does not help end-user communities including those in clinical medical practice, public health or those studying the ecology of the bacteria.

Collectively, in view of the inadequate genetic evidence supporting the genus split and the biological features shared between RF and LB group spirochaetes, at present we strongly oppose the proposed division of the genus *Borrelia*. This division complicates an already complicated situation which will serve only to lead to further confusion among scientists, clinicians, public health authorities and the general public. Taken together, we believe that such a change is inadvisable based on currently available biological and clinical evidence, and therefore respectfully request that it be repealed.

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